Table S1. sGCβ1 mutants, expression vectors, and DNA primers used in this study.

	Mutant \ Vector	pCMV5, pET20b (rat sGC, rat TC-sGC)	pMAL (bovine sGC)
PAS domain mutants	Δ284-301	CTTTGTACTGAGAAGCAAGGAAGGGAGCTGC CTCCGTCTCAAAGGCCAAA	GAGAAGCAAGGAAGGGAGCTGCTTACGGCTCA
	Δ265-271	CTCTCTGGTCCGCCCTCATATTGACATCAGTAT CAATACCGTCTTTGTACTGAGAAGCAAGG	CGTCCTCATATTGACATCAGTATCAATACAGTCTTC GTGTTG
	R335S/R336S/D342S	CTTGGATGACCTAACAAGCAGCGGCCTGTACC TGAGTAGCATCCCTCTCCATGATG	CCTGGACGACCTGACCAGCAGCGGGCTGTACCTGA GTAGCATCCCTCTGCACGATG
	L340D/I343D/L345D	CCTAACAAGAAGAGGCCTGTACGATAGTGACG ACCCTGACCATGATGCTACACGAGACCTG	ACCCGGCGAGGGCTGTACGATAGTGACGACCCTG ATCACGATGCCACTCGGGAC
	H266A/H271A/I272D	CCTCATATTGACATCAGTTTCGCCGGGATTCTT TCAGCCGACAATACCGTCTTTGTACTGAGAAG	CCTCATATTGACATCAGTTTCGCTGGAATCCTCTCA GCCGACAATACAGTCTTCGTGTTGAGAAG
	L269D/I272S/V275D	TCAGTTTCCACGGGATTGATTCACACAGCAATA CCGACTTTGTACTGAGAAGCA	TCAGTTTCCATGGAATCGACTCACACAGCAATACA GACTTCGTGTTGAGAAGCA
	E291A/E295A/L296D	CTGGATGTTGAGAAACTTGCATGTGAGGATGC AGATACTGGGGCAGAGATTAGCTG	GGATGTAGAGAAATCAGCGTGTGAGGATGCGGAT ACGGGCACTGAAATCAGCTGC
	L333D/L338D/L340D	AGTGTGATGAACTTGGATGACGATACAAGAA GAGGCGATTACGATAGTGACATCCCTCTCCAT GATGCTAC	ATGAACCTGGACGACGATACCCGGCGAGGGGATT ACGATAGTGACATCCCTCTGCACG
Heme Binding mutants	H105F	CCTCGACGCCCTGTTCGACCACCTCGCC	
	Y135A R139A	GGGCTCATTCTGCACGCCTACTCGGAAGCAGA GGGGCTTCAGGA	

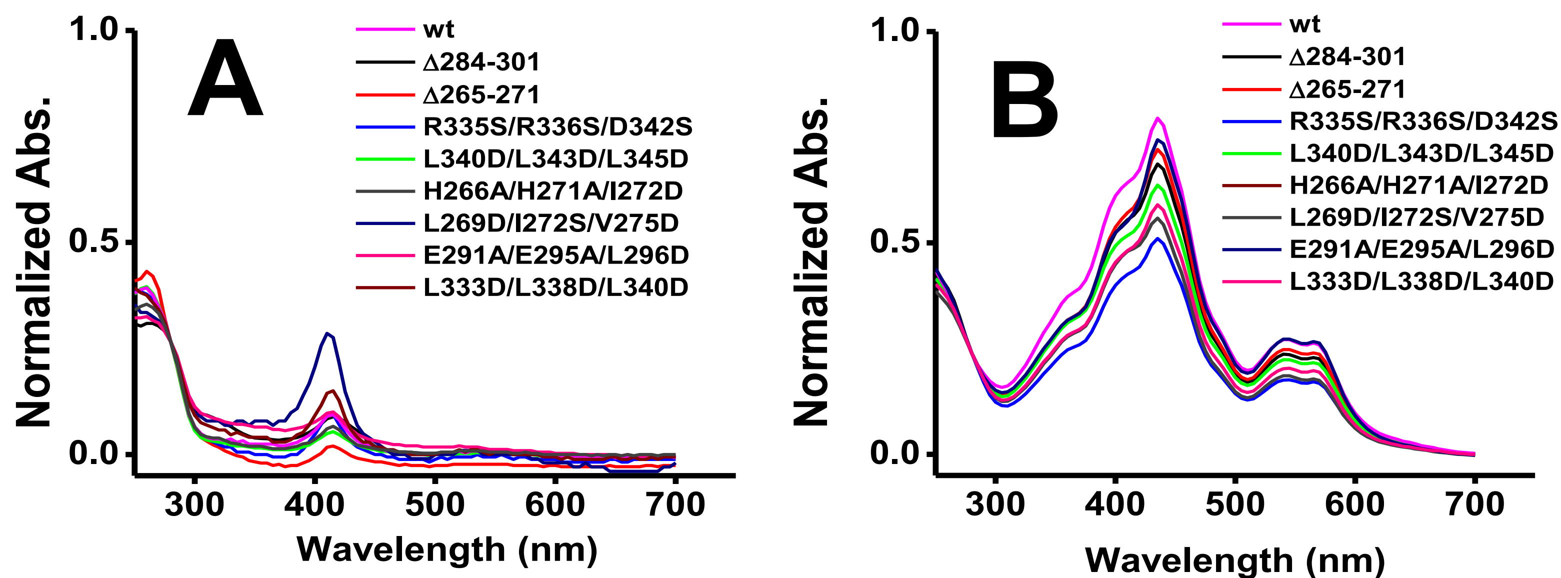

Fig. S1. ***UV-Vis spectrum of purified recombinant sGCβ(1-358) proteins.*** Spectra are normalized to the absorption values at 280 nm. *Panel A,* spectra of proteins after purification. *Panel B,* spectrum of proteins after undergoing heme reconstitution.

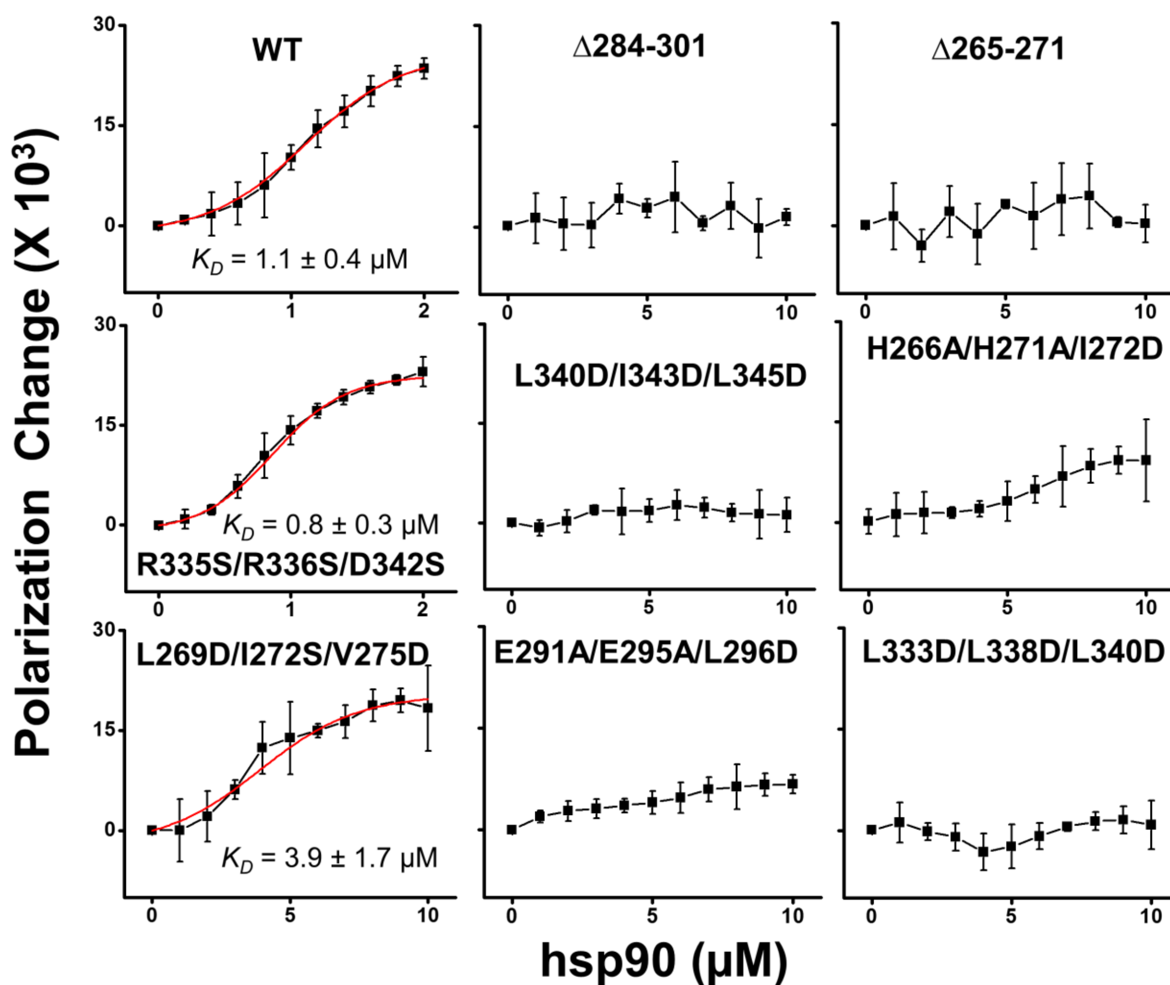

Fig. S2. ***Binding interactions of purified apo-sGCβ1(1-358) mutant proteins with FITC labeled hsp90.*** Each indicated FITC-labeled hsp90 (0.5 µM) was titrated with increasing concentrations of sGCβ proteins and the residual polarization change was recorded and plotted. The points depict mean ± S.D. for three samples, and are representative of two independent experiments. Red lines are computer fits derived using a single site binding equation, and gave the indicated estimates for kd.

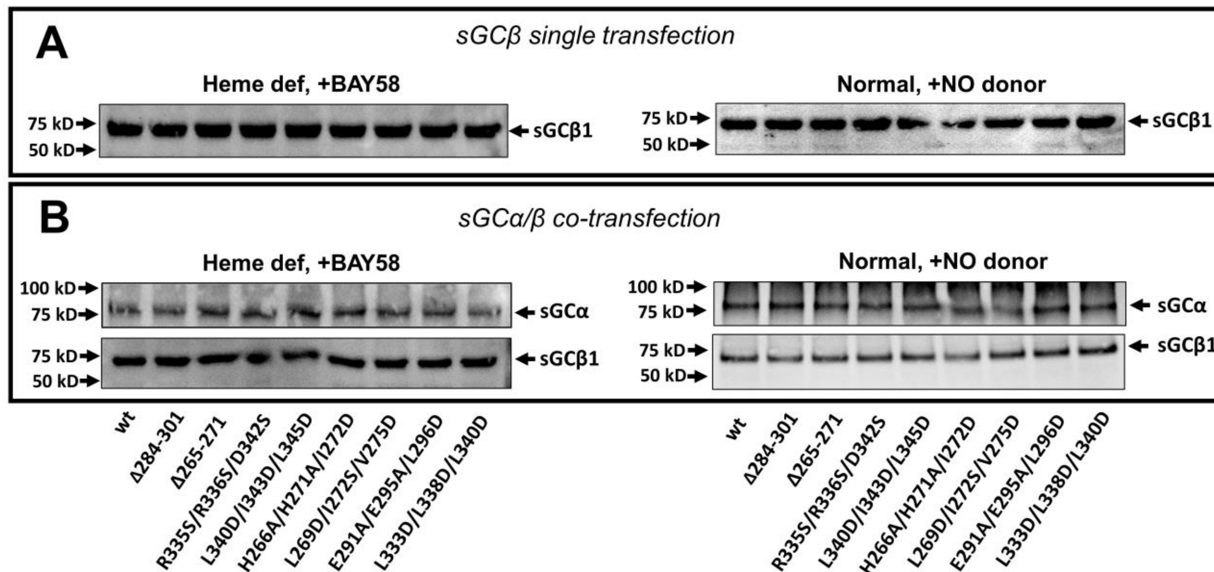

Fig. S3. ***Expression levels of sGCβ1(1–619) proteins in cell supernatants used for guanylyl cyclase activity quantification.*** *Panel A* shows a representative Western blot indicating expression levels of the sGCβ proteins in COS-7 cell that were only transfected with sGCβ constructs. *Panel B* shows a representative Western blot indicating expression levels of sGCβ proteins and sGCα in COS-7 cell that transfected with both sGCα and sGCβ constructs. The analysis used the same supernatants that were used in Fig. 5 A and B.

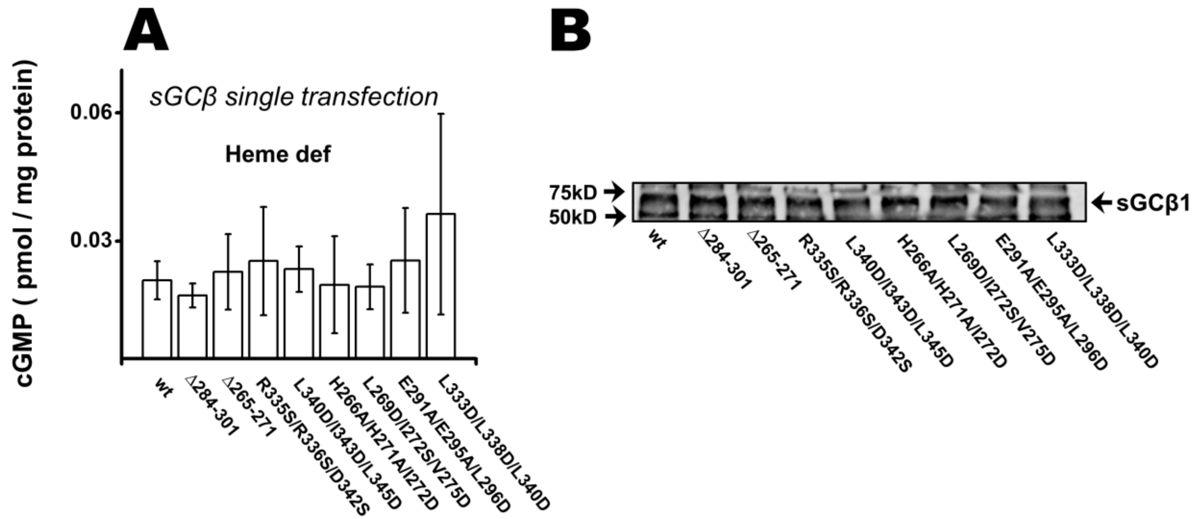

Fig. S4. ***NO-dependent guanylyl cyclase activity of the sGCβ1(1-619)-containing supernatants from heme-depleted cells.*** COS-7 cells were cultured in heme-depleted conditions (heme def) and transfected to express each sGCβ1(1-619) protein. Their supernatants were collected and used for GTP cylcase activity measurements in response to the NO donor NOC18. *Panel A* compares the activities. Values are the mean ± SD of three measurements, and representative of two experiments each. *Panel B* shows a representative Western blot comparing the expression levels of the sGCβ proteins in the supernatants (equal protein loaded).

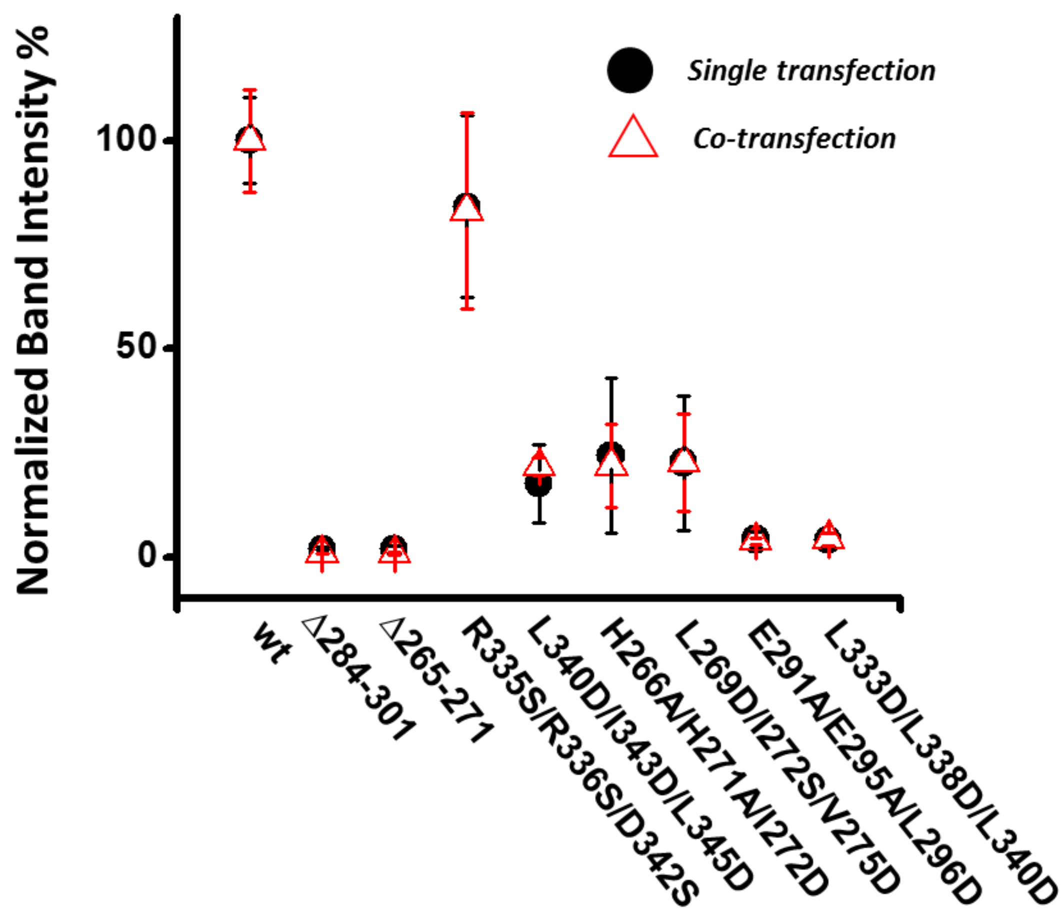

Fig. S5. ***Effect of sGCa co-expression on the hsp90 association levels of the sGCβ1(1-619) proteins expressed in heme-depleted cells.*** The figure plots the normalized hsp90 band intensity levels that are reported in Figs. 3 and 6. Band intensities were normalized relative to the hsp90 band intensity for wild type *sGCβ1(1-619)* under the two different culture conditions.